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WITNESS my hand this
Seventeenth day of September 2002

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ORIGINAL

PROVISIONAL SPECIFICATION FOR AN INVENTION ENTITLED:

Invention Title: Regulation Of Cell Differentiation And Cell Migration.
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The Invention is described in the following statement:

Regulation Of Cell Differentiation And Cell Migration

FIELD OF THE INVENTION

The present invention relates to the use of a method to adjust the behaviour of cells. In particular the invention is concerned with the regulation of cell differentiation and cell migration.

BACKGROUND OF THE INVENTION

When tissues are growing or undergoing repair, cells proliferate and migrate. Growth factors induce cells to replicate, to increase the size of the population of cells that migrate to the appropriate site. During this process cells sequentially attach (adhere) and detach (de-adhere) from surrounding cells and extracellular matrix. Migrating cells also secrete extracellular matrix to which they can adhere, as well as proteolytic enzymes that degrade the extracellular matrix from which they detach.

In many biological processes, cells change or differentiate from one cell type to another in response to growth factor polypeptides and glycoproteins. In mammals, those growth factors may either originate from the progenitor cell undergoing differentiation (autocrine mechanism) or from neighbouring cells (paracrine mechanism). These biological processes include those that occur during normal mammalian development in which cells of different types in the conceptus change into other cell types that form the placenta, tissues and organs of the embryo, the fetus, and eventually the adult. Similar processes occur in adult mammals, for example during tissue repair following injury and in cancer.

The differentiation pathway for some cell types includes migration from one site to another. This can include invasion by the migrating cells into a tissue or organ comprised of or constructed by cells of other types or lineages.

Cells of all types proliferate and migrate during organogenesis and to increase the size of the tissue during normal development and growth in embryonic/fetal life and throughout childhood.

Examples of cells which migrate include cytotrophoblast cells from the early embryo and placenta; endothelial cells migrate to form new blood vessels (angiogenesis) during growth and repair of tissues as in pathological conditions including diabetic retinopathy, rheumatoid

arthritis and cancer; fibroblasts and keratinocytes migrate to repair injured skin, chondroblasts migrate to repair cartilage; osteoblasts and osteoclasts migrate during bone repair; epithelial cells, endothelial cells and fibroblasts migrate during restitution of the endometrium following menstruation; and cancer cells migrate during tumour metastasis.

Embryonic stem cells are pluripotent cells derived from the inner cell mass of the early mammalian embryo from which all cell types of the embryo and all endodermal and mesodermal cells in the extra-embryonic tissues are derived.

Adult stem cells are pluripotent cells found in all mammalian tissues from which all cell lineage types of the body may differentiate.

Cytotrophoblast cells are derived from extra-embryonic ectodermal cells of the conceptus, which comprise the trophectoderm of the blastocyst. Cytotrophoblast cells migrate into the endometrium of the maternal uterine decidua to form the placenta. Invasion of the maternal decidua by cytotrophoblast cells is terminated by fusion of cytotrophoblast cells to form multinucleate cells called placental bed giant cells.

Cancer cells are derived from normal cells that do not undergo typical differentiation. They may invade and migrate to adjacent and remote tissues.

Under specific conditions all of these cell types are able to synthesise and secrete insulin-like growth factor II (IGF-II).

SUMMARY OF THE INVENTION

The present invention is predicated on the discovery of certain interactions between cellular growth factors and opposing actions that control migration or invasion of cells in various cellular environments.

Therefore, according to a first aspect of the present invention, although this need not be the broadest nor indeed the only aspect of the invention there is provided a method of regulating cellular differentiation/migration consisting of adjusting levels of IGF-II available for binding to the cation-independent mannose-6-phosphate (CIM6P) receptor.

We have discovered that IGF-II and latent transforming growth factor-β (TGF-β), the inactive precursor of TGF-β, compete for binding to the CIM6P receptor. IGF-II prevents latent TGF-β binding to the CIM6P receptor. It is known that binding of latent TGF-β to the CIM6P

receptor leads to the production of active TGF-β by the urokinase-type plasminogen activator system (Godar et al, 1999). It follows that IGF-II prevents activation of latent TGF-β. Cells that produce sufficient amounts of IGF-II or cells that are exposed to sufficient amounts of IGF-II therefore cannot convert latent TGF-β into its active form by the urokinase-type plasminogen activator system that forms a complex with the CIM6P receptor on the surface of the cell.

The present invention therefore offers a method of regulating and directing cellular differentiation based on the interaction between IGF-II, latent TGF-β and the CIM6P receptor. The inhibition of activation of TGF-β by IGF-II applies to all mammalian cells that have CIM6P receptors and urokinase-type plasminogen activator receptors.

IGF-II is produced by many migratory cell types and promotes their migratory and invasive behaviour. It is known that TGF-β promotes terminal differentiation and inhibits cell division or cell replication in a variety of cell types, including for example epithelial and mesenchymal cells in the lung (Warburton et al, 2000), skeletal muscle cells (Husmann et al, 1996) and cytotrophoblast cells (Irving & Lala, 1995).

We have discovered that IGF-II binding to the CIM6P receptor prevents local activation of TGF-β. In the case of cytotrophoblast cells, for example, IGF-II thereby prevents migratory or invasive mesenchymal-type cytotrophoblast cells from differentiating into non-migratory or non-invasive giant cell types.

Competition for binding the CIM6P receptor is dependent on the concentration of IGF-II and the concentration of latent TGF-β in the vicinity of cells that have CIM6P receptors. The CIM6P receptors on the surface of cells that produce sufficient quantities of IGF-II or are exposed to sufficient amounts of IGF-II are unable to bind latent TGF-β. In this way IGF-II prevents cells from activating latent TGF-β. In the presence of sufficient IGF-II these cells therefore maintain their mesenchymal type and migratory activity.

Removal of IGF-II permits these cells to activate latent TGF-β. The action of TGF-β converts these cells into non-replicating and non-migratory types.

It will be appreciated that in some situations the promotion of cell differentiation and cell migration is highly desirable, whereas in other situations cell differentiation and migration are harmful to the organism or otherwise undesirable.

Thus, in a first preferred form of the invention there is provided a method of promoting invasive and migratory cell behaviour by exposing said cell to elevated levels of IGF-II, such that the cell CIM6P receptors are unable to bind latent TGF-β.

Conditions in which treatment with insulin-like growth factor II (IGF-II) is beneficial include:

Reproduction in Humans

1. Insulin-like growth factor II treatment of embryos promotes conversion of trophectoderm cells into cytotrophoblast cells thereby increasing the success of implantation of embryos into the uterine decidual endometrium, thereby increasing the success of formation of a viable placenta, and thereby improving the rate of successful pregnancy.

2. Addition of insulin-like growth factor II to embryos produced by in vitro fertilisation techniques can be used to treat infertility.

3. Treatment of pregnant women or their embryos with insulin-like growth factor II can be used to prevent recurrent spontaneous miscarriage.

4. Treatment of pregnant women or their embryos with insulin-like growth factor II can be used to treat and prevent pre-eclampsia.

Diabetic Nephropathy

5. Treatment of diabetic patients with IGF-II can be used to prevent fibrosis in the kidney induced by TGF-β.

Atherosclerosis, Thrombosis and the Prevention of Stroke NB get rid of underlining

6. Treatment of patients with atherosclerosis and/or thrombosis with IGF-II can be used to prevent atherosclerosis, thrombosis, and stroke and accelerate clot resolution.

Embryonic and Adult Stem Cell Differentiation

7. Treatment of embryonic stem cells or adult stem cells with insulin-like growth factor II promotes their differentiation into mesodermal/mesenchymal cell types, including haematopoietic precursor cells and muscle precursor cells, including cardiac muscle cells.

In a second preferred form of the invention there is provided a method of inhibiting cell division, cell migration and promoting terminal differentiation behaviour by exposing said cell to reduced levels of IGF-II, such that the cell CIm6P receptors are able to bind latent TGF-β and thereby promote the activation of TGF-β.

Conditions in which treatment resulting in reduced binding of insulin-like growth factor II (IGF-II) to CIM6P receptors is beneficial include

Cancer

8. Treatment of cancers, including liver, ovarian, prostate and breast cancers, with inhibitors of IGF-II action on CIM6P receptor (for example soluble CIM6P receptor or fragments thereof) will prevent tumour metastasis.

Diabetic Retinopathy

9. Treatment of the eye with inhibitors of IGF-II action on the CIM6P receptor (for example soluble CIM6P receptor or fragments thereof) will prevent diabetic retinopathy.

Rheumatoid Arthritis

10. Treatment of arthritic joints with inhibitors of IGF-II action on the CIM6P receptor (for example soluble CIM6P receptor or fragments thereof) will prevent damage to the joint.

Embryonic and Adult Stem Cell Differentiation

11. Treatment of embryonic stem cells or adult stem cells with inhibitors of IGF-II action on the CIM6P receptor (for example soluble CIM6P receptor or fragments thereof) will promote their differentiation into epithelial cell types, including neurones or their precursors.

The invention is, in a further aspect exemplified by the use of not only IGF-II in its basic form, but also to naturally occurring precursors of and isomers of insulin-like growth factor II. Similarly, the treatments identified above may be carried out using also synthetic analogues of insulin-like growth factor II that have altered ability to bind to type-1 IGF receptors, altered ability to bind to insulin receptors, altered ability to bind to IGF-binding proteins and increased ability to bind to cation-independent mannose-6-phosphate receptors (also known as CIM6P receptors, type-2 IGF receptors and Igf2 receptors).

DIAGNOSTICS

Still further aspects of the invention are concerned with diagnostic uses of IGF-II and the recognition that variation in levels of IGF-II in certain cellular environments enables predictions to be made concerning the differentiation/migration behaviours to be expected from the cells.

12. Thus, in accordance with the invention measurement of the composition of and the sequence of nucleotides in the deoxyribonucleic acid within and near the insulin-like growth factor II gene in human embryos can be used to diagnose their ability to establish successful healthy pregnancy.

13. Similarly, measurement of the amount of messenger ribonucleic acid transcribed from the insulin-like growth factor II gene in human embryos can be used to diagnose their ability to establish successful healthy pregnancy.

14. Measurement of the amount of insulin-like growth factor II protein secreted by human embryos can be used to diagnose their ability to establish successful healthy pregnancy.

15. Measurements as described in 12, 13 and 14 above performed on a specimen obtained from either or both biological parents of a human embryo can be used to diagnose their ability to establish successful healthy pregnancy.

It will be apparent that similar considerations apply to the use of the invention to other mammalian species, such as the horse, cow, sheep, goat and pig.

Insulin-like Growth Factor II

A general example of the action of IGF-II in regulating cellular differentiation/migration is provided by examination of placentation. IGF-II is produced by cytotrophoblast cells. These cells are known to have receptors able to bind IGF-II (Rebourcet et al, 1998). These receptors include type-1 IGF receptors, which bind IGF-I, IGF-II and insulin. In many cell types, binding of IGF-I, IGF-II or insulin to the type-1 IGF receptor promotes cell division, also alternatively known as mitosis, replication, multiplication or proliferation.

Cytotrophoblast cells have another receptor type that binds IGF-II very well, binds IGF-I extremely poorly and does not bind insulin. This receptor has been called the type-2 IGF receptor (Rebourcet et al, 1998). This receptor also binds certain glycoproteins that contain

mannose-6-phosphate and has also been called the cation-independent mannose-6-phosphate (CIM6P) receptor. Competition between IGF-II and latent TGF-β for binding to the CIM6P/type-2 IGF receptor underlies the action of IGF-II in regulating the ability of a cell to undergo migratory or non-migratory behaviours in any environment.

In the human placenta, IGF-II is most abundantly produced by cytotrophoblast cells that have migrated furthest into the maternal decidual endometrium (Irwin et al, 1999). IGF-II is commonly produced by tumour cells, including for example those derived from prostate cancer (Xu et al, 1999), liver cancer (Kim et al, 1998), kidney cancer (Nonomura et al, 1997), cervical cancer (Mathur, Mathur & Young, 2000), muscle cancer (Pedone et al, 1994) and breast cancer (Bates et al, 1995; Ybelle, Vu & Hoffman, 1996). IGF-II is also synthesised by cells in and derived from embryos (Lighten et al, 1997).

Treatment with IGF-II has diverse effects on a variety of cells, including embryonic stem cells (Morali et al, 2000), cytotrophoblast cells from the placenta (Hamilton et al, 1998) and cancer cells (Khandwala et al, 2000).

IGF-II promotes migration of cytotrophoblast cells by a mechanism that has hitherto been unknown (Hamilton et al, 1998).

Transforming growth factor-β

Cytotrophoblast cells have TGF-β receptors able to bind TGF-β (Schilling & Yeh, 2000). Treatment of cytotrophoblast cells with TGF-β promotes their fusion to form non-migratory multinucleate giant cells (Morrish et al, 1998).

Active TGF-β, capable of binding to TGF-β receptors, is derived by proteolytic conversion from inactive latent TGF-β, which does not bind to TGF-β receptors. Latent TGF-β is produced by maternal uterine decidual cells and by cytotrophoblast cells (Graham & Lala, 1991).

Latent TGF-β is known to bind to the CIM6P receptor. Latent TGF-β can also bind to CIM6P receptors that are associated in a complex with plasminogen and urokinase-type plasminogen activator receptors.

It is known that urokinase-type plasminogen activator converts latent TGF-β into active TGF-β by the catalytic action of urokinase-type plasminogen activator when bound to the complex

formed by the simultaneous association of urokinase-type plasminogen activator receptor, plasminogen and latent TGF-β with the CIM6P receptor (Godar et al, 1999). Cytotrophoblast cells are known to have urokinase-type plasminogen activator receptors (Floridon et al, 1998).

Cation-independent mannose-6-phosphate receptor

According to the prior art, binding of IGF-II to the CIM6P receptor is a mechanism that removes and degrades IGF-II. According to the prior art, the CIM6P receptor competes with the type-1 IGF receptor for IGF-II binding. IGF-II binding to the type-1 IGF receptor promotes cell division and replication. According to the prior art, the CIM6P receptor reduces the amount of IGF-II able to bind to the type-1 IGF receptor and thus reduces the ability of cells to proliferate or replicate in response to IGF-II (Kornfeld, 1992; Scott & Weiss, 2000). According to the prior art, the CIM6P receptor prevents IGF-II from promoting cell replication. According to the prior art, IGF-II binding to the CIM6P receptor does not produce a direct biological response within the cell upon whose surface the CIM6P receptor is located (Odell & Day, 1998).

DESCRIPTION OF PREFFERED EMBODIMENT

The use of regulation of cell differentiation and migration are aptly demonstrated with reference to the use of IGF-II in early pregnancy to treat both implantation failure and recurrent spontaneous miscarriage.

During pregnancy cytotrophoblast cells that are originally derived from the trophectoderm of the blastocyst and which subsequently form the placenta remodel the endometrium. The placenta is the active interface between maternal and fetal tissues and is the organ through which exchange of soluble materials between fetus and mother occurs.

The embryo establishes its attachment with the endometrium through the actions of migratory cytotrophoblast cells. They secrete metalloproteinases that degrade the extracellular matrix of the endometrium, permitting their invasion. These invasive cells modify the endometrial spiral arterioles to provide access for the placenta to the maternal blood supply, the source of all matter for embryonic and fetal growth. Cytotrophoblast cells form columns that extend deep into the endometrium. The first wave of invasion of the endometrium by cytotrophoblast cells occurs between 6 and 12 weeks of pregnancy in humans (Pijnenborg et al, 1983). This is the period when most miscarriages occur. IGF-II is abundantly produced early in pregnancy by cytotrophoblast cells at the leading edge of the

invasive front of the cell column. IGF-II promotes the invasive behaviour of cytotrophoblast cells. Latent TGF-β is produced by decidual cells of the maternal endometrium as well as by cytotrophoblast cells. TGF-β promotes fusion of cytotrophoblast cells, an essential step in their differentiation to form mature components of the placenta (Morrish et al, 1998). TGF-β also induces synthesis and secretion of tissue inhibitors of metalloproteinases (TIMPs) by cytotrophoblast cells (Lala & Graham, 1990), thus preventing cytotrophoblast cells from degrading the extracellular matrix necessary for their migration.

We claim that early pregnancy loss due to failure to establish a viable placenta is caused by IGF-II deficiency in the IGF-II/TGF-β/CIM6P receptor system of cytotrophoblasts. Further, we claim that failure of the embryo to successfully implant on the uterine endometrium of the mother is due to insufficient production of IGF-II by cytotrophoblast cells derived from the trophectoderm of the blastocyst, leading to reduced autocrine stimulation of invasion of the endometrium by cytotrophoblast cells. We claim that removal or lack of IGF-II induces terminal differentiation and fusion of cytotrophoblasts.

We have discovered that recurrent spontaneous miscarriage (diagnosed between the 6th and 12th weeks of pregnancy) is caused by deficiency in IGF-II production of cytotrophoblast cells leading to premature fusion before sufficient colonisation of the endometrial decidua has been achieved to establish a healthy placenta.

IGF-II can be used to treat both implantation failure and recurrent spontaneous miscarriage.

We have discovered that IGF-II and latent TGF-β, the precursor of TGF-β, compete for binding to CIM6P receptors in human placenta. Latent TGF-β binding to CIM6P receptor is known to lead to its proteolytic conversion by plasmin into active TGF-β by the complex formed between urokinase-type plasminogen activator (uPA), uPA receptor and plasminogen (Godar et al, 1999). It is known that cytotrophoblast cells have CIM6P receptors (Rebourcet et al, 1998) and uPA receptors (Floridon et al, 1999). It is obvious therefore, that secretion of IGF-II by cytotrophoblast cells maintains their invasive state by preventing local activation of TGF-β, the initiator of their terminal differentiation into non-replicating and non-migratory cells. It is known that invasive cytotrophoblast cells produce IGF-II in abundance (Irwin et al, 1999). We show herein that IGF-II and latent TGF-β compete for binding to the placental CIM6P receptor. It is known that activation of TGF-β from its latent form is a prerequisite for its promotion of cytotrophoblast cell fusion and terminal differentiation. We claim that IGF-II inhibits terminal differentiation of cytotrophoblast cells. We claim that IGF-II promotes cytotrophoblast cell invasion of the decidua.

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